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Testing of Mode of Action of Potentail Anti-fouling and Fouling-release Coatings for Microfouling in Marine Systems.

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**13. ABSTRACT** *(Maximum 200 words)*

Tryptophan fluorescence is a non-destructive measure of bacterial biomass utilized for the determination of coating performance over time. Concurrent bioluminescence measurements give an indication of the metabolic activity of the same cell population providing an indication of sub-lethal stress. With this dual method, biofilm density and activity from cells attached to control and test surfaces can be monitored locally and in real time, in the laminar-flow environment. The destructive technique of comparing phospholipid fatty acid ratios provides a way to measure directly the "health" of the biofouling community and avoid tedious plate counts. Control studies have shown that a fluorescence ratio of < 0.65 demonstrates an antifouling (AF) effect when coating values are normalized to stainless steel. There does not appear to be a significant difference between the AF efficacies of different non-toxic polymers. What is apparent is that the best results are obtained with the combination of a fouling release type polymer surface and an AF additive such as C9211. The phospholipid fatty acid ratios indicating stress in bacterial population were shown to increase in biofilms exposed to coatings with C9211 relative to coatings without the compound.

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# FINAL REPORT

Grant #: N00014-94-1-0961 R&T Code: biofl-010

PRINCIPAL INVESTIGATOR: Dr. David C. White

INSTITUTION: University of Tennessee

GRANT TITLE: Testing of Mode of Action of Potential Anti-fouling and Fouling-release Coatings for Microfouling in Marine Systems

PERIOD OF PERFORMANCE: 1 May 1994 - 31 April 1996

OBJECTIVES: 1) To rank the efficacy of antifouling (AF) and fouling-release (FR) coatings supplied by ONR contractors and from commercial sources to inhibit microfouling. 2) To compare differences in the molecular structure of polymers (bound vs. tethered chains, PDMS: filler ratios, modulus etc.) on coating performance against bacterial biofilms. 3) To determine the effect of various flux rates of AF compounds on biofilm attachment/growth and whether the compounds are active when bound to a surface or when released into the bulk solution. 4) Utilize the tandem-flow membrane reactor to distinguish between cell recruitment from the bulk-phase and cell surface growth: and determine what effects the candidate AF compounds have on these processes

APPROACH: Test coatings were inserted into a laminar-flow cell system inoculated with the bioluminescent bacterium *Vibrio harveyi*. Biofilm biomass was monitored non-destructively by tryptophan fluorescence, and cellular metabolic activity was monitored by bioluminescence. All measurements were normalized to a biofilm population attached to a stainless steel surface located directly upstream of the coating. Biofilm accumulation was measured over a 4 d period under low-flow, low-shear conditions to determine the AF efficacy of each coating. The flow rate of the bulk liquid medium was increased to a calculated shear stress of 330 dynes $cm^{-2}$ for 15 min and the percentage of biofilm biomass stripped was measured to determine FR efficacy.

To determine the effect of zosteric acid (ZA) on suspended vs. attached cell growth; *V. harveyi* was exposed to ZA in batch cultures and introduced in the bulk phase of the laminar flow system during *V. harveyi* biofilm formation. The free acid form of ZA was utilized in concentrations of 0, 0.05, 0.1, 0.5 and 1.0 g $ml^{-1}$.

A tandem-flow membrane reactor was developed utilizing a permeable membrane filter to separate two flow channels. Experiments were conducted by introducing cells into the top chamber and monitoring biofilm development while controlling the concentration of ZA in the lower chamber.

ACCOMPLISHMENTS: The following coatings were tested for both AF, and FR efficacy: 1)Commercial Coatings- Intersleek, MIA, RIA, RTH, DRC; 2) PDMS coatings DO2 through DO7, as well as a series of PDMS polymers with differing polyether additives, and a series of copolymers with C9211from Dow Corning; 3) fluorinated and non-fluorinated polyurethane from Dr Toby Chapman of the University of Pittsburgh; 4) three perfluorooctylacrylate coatings with differing levels of fluorination from NCCOSC and; 5)three PDMS coatings with differing ratios of vinyl double bonds to silane hydrogens. The AF potential of Elorisan$^{R}$ was tested by growing *Vibrio harveyi* biofilms in the presence of 0.01 and 0.1 mg.ml added to the bulk media Tryptophan fluorescence and bioluminescence were monitored on-line for 4 days, and cell densities were determined as an endpoint measurement.

Conclusions: There does not appear to be a significant difference between the AF efficacies of different non-toxic polymers against bacteria. What is apparent is that the best results are obtained with the combination of a fouling release polymer surface and an AF additive such as C9211.

The phospholipid fatty acid ratios indicative of stress in bacteria were shown to increase in biofilms exposed to coatings with C9211 relative to coatings without the compound. This technique provides a way to measure directly the "healthy" of the biofouling community thus avoiding tedious plate counts

SIGNIFICANCE: Tryptophan fluorescence is a non-destructive measure of biomass utilized for the determination of coating performance over time. Control studies have shown that a fluorescence ratio of $< 0.65$ demonstrates and AF effect when coating values are normalized to stainless steel. Concurrent bioluminescence measurements give an indication of the metabolic activity of the same cell population providing an indication of sub-lethal stress. With this method, biofilm density and activity from cells attached to control ant test surfaces can be monitored locally in the laminar-flow environment. To date a total of 55 coatings have been tested for AF efficacy and 41 coatings for FR performance.

PUBLICATIONS AND ABSTRACTS: (for total granting period)


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